

Technical Appendix

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ELISA Data Analysis Example

Plate 1: 7/7/07

Raw data is imported as a text file from the platereader software.

RAW DATA

	1	2	3	4	5	6	7	8	9	10	11	12
Dilution:	MPV Immune Subject			VV Immune Subject			Naive Subject					
	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed			
30	0.754	2.708	4.000	0.398	0.436	4.000	0.140	0.181	0.121			
90	0.205	0.855	2.496	0.187	0.179	1.633	0.070	0.088	0.070			
270	0.139	0.418	1.274	0.123	0.122	0.651	0.060	0.064	0.050			
810	0.099	0.210	0.557	0.077	0.084	0.252	0.050	0.059	0.045			
2430	0.073	0.111	0.229	0.070	0.064	0.146	0.017	0.020	0.015			
7290	0.056	0.073	0.119	0.072	0.086	0.092	0.006	0.007	0.005			
21870	0.051	0.058	0.066	0.059	0.091	0.087	0.002	0.002	0.002			
Blank	0.046	0.047	0.050	0.044	0.045	0.048	0.050	0.052	0.051	0.050	0.052	0.051

Average Blank:

AVERAGE(D15:O15)

0.049

The average blank is calculated from 12 wells.

Adjusted OD values are calculated by subtracting the Average Blank OD from each raw data value.

Values between OD 0.05 and 1.5 (those points in the linear portion of the dilution curve) are selected to calculate the final titer.

BLANK SUBTRACTED

Raw OD - D18

Select values 1.5 > x > 0.05 (yellow)

	1	2	3	4	5	6	7	8	9	10	11	12
Dilution:	MPV Immune Subject			VV Immune Subject			Naive Subject					
	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed			
30	0.705	2.659	3.951	0.349	0.388	3.951	0.091	0.132	0.072			
90	0.156	0.806	2.447	0.138	0.130	1.584	0.021	0.039	0.021			
270	0.090	0.369	1.225	0.074	0.074	0.602	0.011	0.015	0.001			
810	0.050	0.161	0.508	0.028	0.035	0.203	0.001	0.010	-0.004			
2430	0.025	0.062	0.180	0.021	0.015	0.097	-0.032	-0.029	-0.034			
7290	0.007	0.024	0.070	0.023	0.037	0.043	-0.043	-0.042	-0.044			
21870	0.002	0.009	0.017	0.010	0.042	0.039	-0.047	-0.047	-0.047			

Values are next log-transformed by calculating the Log10 of the blank-subtracted OD and the dilution values.

LOG BLANK SUBTRACTED

LOG10(Blank Subtracted Value)

LOG10(dilution)

	1	2	3	4	5	6	7	8	9	10	11	12
	MPV Immune Subject			VV Immune Subject			Naive Subject					
	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed			
1.477	-0.152			-0.458	-0.412		-1.042	-0.880	-1.143			
1.954	-0.806	-0.094		-0.861	-0.886							
2.431	-1.047	-0.432	0.088	-1.129	-1.133	-0.220						
2.908	-1.301	-0.794	-0.294			-0.692						
3.386		-1.209	-0.746			-1.013						
3.863			-1.154									
4.340												

Endpoint values are calculated by Excel using the equation of the linear portion of the dilution curve, and transformed to linear values in the final step.

Endpoint titers are visually confirmed by ensuring that the calculated titer is roughly the dilution where blank subtracted OD is expected to equal 0.1 (blue boxes).

Intercept:

Slope:

A490:

log A490

log Dilution

Dilution

(endpoint titer)

	1	2	3	4	5	6	7	8	9	10	11	12
	MPV Immune Subject			VV Immune Subject			Naive Subject					
	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed	MPV adsorbed	VV adsorbed	Unadsorbed			
	0.868	1.443	2.229	0.560	0.668	1.774						
	-0.77	-0.78	-0.88	-0.70	-0.76	-0.83						
	0.100	0.100	0.100	0.100	0.100	0.100						
	-1.000	-1.000	-1.000	-1.000	-1.000	-1.000						
	2.42	3.14	3.69	2.22	2.21	3.34						
	262	1390	4875	164	160	2186	27	40	22			

Figure 1. Illustration of ELISA data analysis. Optical density values at 490 nm (OD₄₉₀) values were imported directly into Excel (Microsoft, Redmond, WA) from the ELISA microplate reader (VersaMax; Molecular Devices, Sunnyvale, CA, USA). The average OD₄₉₀ of 12 blank wells (Line 18) was then subtracted from the raw OD₄₉₀ obtained for each sample well. These values were transformed to logarithmic scale to create a log-log curve. We use linear OD₄₉₀ values between 1.5 and 0.05 because these typically fall within the linear portion of the curve and are used to determine the endpoint titer. The slope and intercept of the linear portion of the curve were determined by a standard $y = mx + b$ calculation, and the dilution at which OD₄₉₀ = 0.1 was transformed to a linear value to give a final endpoint titer.